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Oncogenic signalling pathways in benign odontogenic cysts and tumours

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Highlights

- The study of benign neoplasms may reveal relevant aspects of malignant progression
- Oncogenic mutations considered drivers of cancers are reported in benign lesions
- The role of oncogenic mutations seems to be context dependent
- Pathogenic mutations affecting the same pathway are seen in different tumours
- Molecular studies can guide therapeutic approaches towards odontogenic lesions

Abstract

The first step towards the prevention of cancer is to develop an in-depth understanding of tumourigenesis and the molecular basis of malignant transformation. What drives tumour initiation? Why do most benign tumours fail to metastasize? Oncogenic mutations, previously considered to be the hallmark drivers of cancers, are reported in benign cysts and tumours, including those that have an odontogenic origin. Despite the presence of such alterations, the vast majority of odontogenic lesions are benign and never progress to the stage of malignant transformation. As these lesions are likely to develop due to developmental defects, it is possible that they harbour quiet genomes. Now the question arises – do they result from DNA replication errors? Specific candidate genes have been sequenced in odontogenic lesions, revealing recurrent *BRAF* mutation in the case of ameloblastoma, *KRAS* mutation in adenomatoid odontogenic tumours, *PTCH1* mutation in odontogenic keratocysts, and *CTNNB1* (Beta-catenin) mutation in calcifying odontogenic cysts. Studies on these benign and rare entities might reveal important information about the tumorigenic process and the mechanisms that hinder/halt neoplastic progression. This is because the role of relatively common oncogenic mutations seems to be context dependent. In this review, each mutation signature of the odontogenic lesion and the affected signalling pathways are discussed in the context of tooth development and tumorigenesis. Furthermore, behavioural differences between different types of odontogenic lesions are explored and discussed based on the molecular alteration described. This review also includes the employment of molecular results for guiding therapeutic approaches towards odontogenic lesions.

Keywords: Odontogenic tumours; odontogenic cysts; oral cancer; tumorigenesis; benign tumours; oncogenic mutations; oncogenic signalling.

Introduction

The compilation of a Precancer Atlas has been proposed to integrate large-scale research and thus improve the understanding of the events that drive oncogenesis [1]. The study of benign tumours is considered as important as the study of malignant ones, as they may reveal relevant aspects of neoplastic progression [2]. The genomic stability inherent in benign tumours contributes to the identification of remarkably clear genetic fingerprints, pointing to specific molecular alterations that are likely to be responsible for tumorigenesis.

In a similar fashion to malignant neoplasms, benign tumours harbour one or several oncogenic mutations that lead to clonal cell proliferations. Notably, benign tumours share five of the six putative “hallmarks of cancer”, with metastasis being the exception [3,4]. It is intriguing that some benign tumours lack fibrous capsules and can show markedly aggressive behaviour, while others grow no more than a few cubic centimetres inside a thick fibrous capsule. Odontogenic lesions mainly comprise benign cysts and neoplasms of the tooth apparatus, including lesions that can perforate the cortical plate and infiltrate the soft tissues, such as ameloblastomas, and encapsulated lesions with indolent behaviour, such as adenomatoid odontogenic tumour (AOT). These benign tumours can harbour oncogenic genetic alterations that were previously considered as the drivers of specific, invasive cancers originated in other organs, such as melanomas, lung and colorectal adenocarcinomas [5]. It is not clear why the majority of the benign conditions never progress to cancer, even in the presence of pathogenic mutations. In this review, we used the

odontogenic cysts and tumours as the models to discuss the context-specific role of oncogenic signalling pathways, which are important for understanding tumour biology in a broader perspective.

The morphology of odontogenic lesions resembles the stages of tooth embryonic development (i.e. odontogenesis) (Figure 1), while the molecular mechanisms associated with odontogenesis have been associated with the pathobiology of such entities. While in ameloblastomas, the gene expression profile reflects differentiation from the dental lamina towards the cap/bell stage of tooth development, odontogenic keratocysts (OKCs) show differentiation toward keratinocytes [6]. Every step of normal odontogenesis is regulated by BMP, FGF, Shh and Wnt cell signalling pathways [7]. During the past years, pathogenic gene mutations affecting downstream signalling cascades of these signalling pathways were described in odontogenic lesions, including *BRAF* mutation in ameloblastoma, *KRAS* mutation in AOT, *PTCH1* mutation in OKC, and *CTNNB1* (Beta-catenin) mutation in calcifying odontogenic cyst (COC).

In this review, we discuss the mutation signatures of some odontogenic cysts and tumours, together with their significance in cell signalling pathways in the context of odontogenesis and tumorigenesis. Furthermore, insights into the histopathogenesis and behavioural differences between the lesions are brought into light on the basis of the described molecular alterations.

The MAPK pathway, ameloblastoma and adenomatoid odontogenic tumour

The prototypical MAPK cascade, the Ras-Raf-MEK-ERK, is commonly dysregulated in several human cancers. In this pathway, the activation of a family of cell-surface tyrosine kinase receptors triggers the proteins of the Ras superfamily of small GTPases, which includes molecules encoded by *KRAS, HRAS,* and *NRAS* [8]. Activated Ras binds and activates Raf kinases, encoded by *BRAF*, *RAF1,* and *ARAF*. Raf phosphorylates and activates MEK1/2, which in turn phosphorylates and activates ERK-1/2. ERKs phosphorylate a vast array of substrates both in the cytosol and in the nucleus [9] (Figure 2), including receptors, protein kinases, signalling effectors, and transcription factors. Recently, alterations of prototypical MAPK cascade components were reported in a high proportion of benign odontogenic lesions, mainly in ameloblastoma and AOT [10,11].

The activating mutation BRAFV600E occurs in 60–80% of ameloblastoma cases, regardless of clinical and histopathological variants, including its malignant counterpart, ameloblastic carcinoma (AC) [10,12–15]. Interestingly, MAPK activation had previously been detected by immunohistochemistry in ameloblast-like cells neighbouring the basement membrane of ameloblastomas, as well as in tooth germs [16]. Other odontogenic lesions were also shown to be BRAFV600E positive, including clear cell odontogenic carcinoma, ameloblastic fibroma, ameloblastic fibrodentinoma, and ameloblastic fibro-odontoma [12,13,17].

Genetic mutations mutually exclusive of BRAFV600E, such as those in *KRAS*, *NRAS, HRAS,* and *FGFR2*, were described in *BRAF* wild-type ameloblastomas [12,14]. Additionally, a high-density whole-genome microarray

analysis showed only a few rare DNA copy-number alterations, mostly affecting genes associated with MAPK activation [18]. These results support the crucial role of the MAPK pathway activation in the pathogenesis of ameloblastomas. Secondary events, such as changes in tumour suppressor genes, may also play a role in cytodifferentiation and tissue structuring of these neoplasms [19,20].

The MAPK pathway is also altered in AOT, which is similar to ameloblastoma – an epithelial odontogenic neoplasm (Figure 2). In 2016, we reported the activating pathogenic mutation KRASG12V in 7/9 AOT samples by carrying out a targeted next-generation sequencing approach in a panel containing ~2,800 COSMIC mutations in 50 genes [11]. Even though ameloblastoma and AOT neoplasms harbour gene mutations affecting MAPK cascade, their clinical behaviours are markedly distinct. While ameloblastoma is locally aggressive and can perforate the cortical bone plate and infiltrate soft tissues, AOT is encapsulated with limited growth potential and very low tendency of recurrence. Notably, both the odontogenic tumours show expression of the MAPK transcriptional targets, cyclin-D1 and c-Myc, which regulate cell proliferation [21–25]. In colorectal cancer, it has been shown that BRAFV600E and KRASG12V oncogenes have a different impact on morphology and invasive phenotype [26]. Furthermore, in contrast to KRASG12V, BRAFV600E caused an increased expression of unique target genes, such as *SOX2* [27]. In lungs, different tumorigenic capacity of oncogenic BRAF and KRAS were reported [28,29]. On this basis, although MAPK pathway is activated in ameloblastoma and AOT, distinct roles of BRAF and KRAS oncogenes within each tumour context, including the cell-of-origin and the

prevalent location at the jaws, may account for the distinct biological behaviour of these neoplasms.

The high prevalence of mutations affecting MAPK signalling pathway in ameloblastoma and AOT indicates that this is likely an early pathogenic event. The evidence that MAPK cascade plays a central role in odontogenesis, including ameloblast development, has shed light on the intimate connection between oncogenic MAPK signalling, ameloblastoma, and AOT tumorigenesis, as we discuss below.

Growth factors, such as FGF and EGF, are the major regulators of the MAPK cascade in many physiological and cellular contexts, including odontogenesis. At the bell stage of tooth development, the downregulation of FGFR-2, a FGF receptor, was reported in the pre-ameloblast cells [30] in association with the cessation of cell proliferation [31]. Curiously, FGFR-2 was found to be over activated by mutation in some ameloblastoma samples [14], suggesting that a sustained hyper-proliferation is mediated by oncogenic FGF signalling. Corroborating these findings, treatment with FGF ligands lead to ameloblastoma cultured cells' increased proliferation through ERK-1/2 signalling, an effect blocked by MEK inhibition [32].

The EGF receptor, erbB-1, is encoded by *EGFR,* and its abnormal function is implicated in tumorigenesis [33,34]. The expression of *EGFR* in ameloblasts varies throughout the developmental stages. It is expressed in the inner enamel epithelium (IEE), which contains proliferative and differentiating cells, while it is expressed in low levels in polarizing ameloblasts, and its expression remains low during the enamel secretion stage [35]. Interestingly,

EGFR is overexpressed in ameloblastomas [10,36], in a similar fashion to other epithelial neoplasms such as head and neck, cervical, and oesophageal cancer [37]. In ameloblastoma cultured cells, *EGFR* knockdown blocked the activity of matrix metalloproteinases (MMPs), and abolished EGF-stimulated cell migration and invasion [38].

In addition to studies with human tumours and *in vitro* experimentation, *in vivo* experiments support that MAPK pathway dysregulation impacts the ameloblasts' function. Activation of MAPK cascade caused by HrasG12V mutation led to compromised proliferation and differentiation of enamel-producing ameloblasts and their precursors in mice incisors [39]. Notably, HrasG12V ameloblasts were shown to be disorganised and with abnormal cell polarity [39], a characteristic shared with ameloblast-like cells in ameloblastoma and AOT. Further analyses supported the conclusion that low levels of phosphorylated ERK-1/2 (i.e. the MAPK downstream effector) might be necessary for the normal ameloblast progenitors to exit the cell cycle and to differentiate [39]. Studies with Hras transgenic mice reported the development of jaw tumours compatible with the diagnosis of odontogenic tumours [40–42].

MAPK-targeted therapy has been tested in ameloblastoma cells *in vitro*. Treatment with erbB-1 monoclonal antibodies (cetuximab and panitumumab) and erbB-1 tyrosine kinase inhibitors (erlotinib, gefitinib, and AG1478) resulted in ameloblastoma cell growth suppression [10]. Conversely, resistance to erbB-1 inhibition was observed in BRAFV600E ameloblastoma cells [10]. Such resistance to ErB-1 inhibition was attributed to nuclear translocation of erbB-1 in certain cancer types [43]. This erbB-1 nuclear localization was observed in

ameloblastomas by Pereira and colleagues [21], raising a concern about an additional resistance mechanism of anti-erbB-1 therapy in ameloblastomas [21]. In addition to *in vitro* testing, BRAF-mutant targeted therapy has already been clinically tested in human ameloblastomas [44,45]. In one case, a patient with metastatic ameloblastoma was treated with a dual therapy with BRAF/MEK inhibition (dabrafenib and trametinib) [44], whereas in the other case, a patient with recurrent ameloblastoma received only dabrafenib [45]. Both the case reports described a reduction in the tumour mass and suggested the possibility of using neoadjuvant and/or adjuvant targeted therapy in a subset of aggressive ameloblastoma undergoing surgery [44,45]. However, there are important adverse effects of this treatment, and its long-term complications include an increase in the chances of developing squamous cell carcinomas.

Shh pathway and odontogenic keratocyst

In mammals, there are three Hedgehog-family members, SHH, IHH and DHH. SHH triggers short and long-range signalling activities [46,47]. The Shh signalling activity takes place after the secreted ligand undergoes a series of post-translational modifications, including a covalent modification by a cholesterol moiety added to its carboxyl-terminal domain and attachment of a palmitic acid group to its amino-terminal end [48]. Cooperation between two transmembrane proteins, Dispatched and SCUBE2, culminates with the SHH release from the surface of the signalling cells [49,50]. The secreted SHH ligand binds to its receptor PTC1, a twelve-pass transmembrane protein, encoded by *PTCH1* [51]. In the absence of its ligand, PTC1 works as a ligand-independent inhibitor of the downstream SMO, a seven-pass transmembrane protein

member of the G-protein coupled receptor superfamily [50,52]. The current concept is that this is not a direct inhibition, as biochemistry assays demonstrated that PTC1 and SMO do not physically interact [53].

The fine-tuning of SHH activity has recently been shown to take place in the primary cilium, a nonmotile microtubule-based organelle present in most mammalian cells, by an as-yet-undefined mechanism [54,55]. Once the repression exerted by PTC1 is released, the resulted increase in ciliary levels of SMO leads to processing of GLI1-3 transcription factors [56–58] (Figure 2). While GLI3 can be proteolytically processed and work both as a repressor or an activator of the pathway, GLI1 and GLI2 act as activators. Overall, the different concentration thresholds of GLI is sufficient to mediate graded Shh signalling [59].

PTCH1 mutation leads to constitutive activation of the Shh pathway, and it is considered as the major mechanism underlining OKC tumorigenesis [60]. OKC occurs in isolation or as part of the nevoid basal cell carcinoma syndrome (NBCCS) (OMIM #109400) [61]. In 1996, *PTCH1* genetic mutation was reported as the underlying cause for NBCCS [62,63]. Subsequently, loss of heterozygosity (LOH) and mutations at *PTCH1* were reported in syndromic and sporadic OKCs [64–66]. Recently, the DNA analysis of the microdissected OKC epithelium revealed that a *PTCH1* genetic alteration is present in ~80% of the sporadic OKC [67]. Activating *SMO* mutations [68],as well as transcriptional and immunohistochemical studies reinforce Shh pathway over activation in human OKC [60,69,70]. In vivo, *Ptch1* knockout and constitutive activation of *Gli2* were reported to drive mandibular OKC-like lesions in mice [71,72]. This evidence

strengthens the link between Shh signalling pathway misregulation and OKC tumorigenesis.

Despite the exciting possibility of *PTCH1* mutations being an OKC signature, LOH at the *PTCH1* loci occurs in other odontogenic lesions such as the orthokeratinized odontogenic cyst and ameloblastomas [73–76]. Also, *PTCH1* mutation was previously described in the calcifying epithelial odontogenic tumour (CEOT) [77]. However, the effects of these genetic alterations in PTC1 function were not determined.

Although OKC was recently reclassified by World Health Organization (WHO) as an odontogenic cystic lesion [78], numerous studies have considered it as a true cystic neoplasm. OKC epithelium has an intrinsic growth potential that is not seen in other cysts, and this lesion presents an elevated recurrence rate of 30% [79]. Moreover, OKC can attain a large size resulting in massive bone destruction [79].

In the past, the concept of “primordial cyst” was used to refer to OKC. The cyst was believed to have a primordial origin, arising from the dental lamina (tooth bud) before enamel formation had taken place, and even by substituting a tooth [80]. A recent study showed that OKC cells harbour expression profiles similar to keratin-producing cells (i.e. keratinocytes) [6]. In this study, Affymetrix whole-genome arrays were used to hybridise total RNA from 12 sporadic OKC. In addition to Shh pathway genes, squamous and terminal epithelial differentiation markers were found to be overexpressed [6]. This study provided molecular evidence to support the concept that OKC originates from fetal oral

epithelium or dental lamina at early stages of odontogenesis rather than the enamel organ (Figure 1).

It is well known that Shh signaling has pivotal roles in early odontogenesis, stimulating epithelial cell proliferation to drive the formation and growth of the tooth bud and increasing epithelial cell survival during the development of the cap-staged tooth germs [81]. One of the most important targets of Shh signalling is *BCL2*, which encodes a pro-survival protein. In human tooth germs, a strong immunoexpression of Bcl-2 occurs in the dental lamina and tooth bud, the most primitive form of odontogenic epithelium [82]. Moreover, Bcl-2 protein was also observed in dental lamina cells even when it is fragmented into cell nests, but it disappears after squamous maturation [82]. Interestingly, Bcl-2 overexpression is a hallmark of OKC epithelium [83] and basal cell carcinoma (BCC) [84]. We propose the hypothesis that during OKC tumorigenesis, Shh misregulation might lead to persistent Bcl-2 production in the primitive odontogenic epithelium, reactivating its proliferative and survival potential. Supporting this hypothesis, mice overexpressing the Shh pathway effector GLI2, under the control of keratin 5 promoter, were shown to develop OKC-like and Bcl-2-expressing BCC-like lesions [72,85]. Although a direct link between Bcl-2 overexpression and Shh pathway misregulation were only analysed in the BCC [85], it is very likely that Bcl-2 overexpression at OKC epithelium line is driven by Shh signalling activation.

Another important gene regulated by Shh signalling in several contexts is the transcription factor SOX-2 [86], which is a marker of stem cells and progenitor cells in epithelial tissues [87]. During tooth development in mammals,

SOX-2 is associated with the epithelial competence of dental lamina, both for the successional tooth formation and the serial addition of molars [88]. SOX-2 expression was also described in dental lamina and in its fragments associated with the developing third and fourth molars [88,89]. Interestingly, this transcription factor is implicated in cell proliferation and self-renewal in the Shh-associated tumours [86,90] and was also reported to be highly expressed in basal and suprabasal epithelial cell layer of OKC [6,91]. These findings support the idea that Shh-associated OKC arises from SOX-2-positive dental cells [6], especially those cells located at the posterior extension of the dental lamina in the mandible, the site most frequently affected by some odontogenic lesions (Figure 3). Likewise, SOX-2-positive cells are linked with odontoma, ameloblastoma, and AC tumorigenesis (Figure 3) [92–94].

As the alteration in Shh signalling is the main feature in OKC, the pathway inhibitors have been tested therapeutically. In OKC primary cultured cells, the use of the SMO antagonist cyclopamine showed a dose-dependent growth arrest of the cells, together with Shh pathway downregulation [95]. A nearly complete resolution of three NBCCS-associated OKC was reported in one case of a 55-year-old man after administration of Shh signalling antagonist GDC-0449 (Vismodegib) [96]. The effects of Vismodegib were later tested in a clinical trial study and an overall OKC size reduction in four out of six NBCCS patients was reported [97].

The Wnt pathway and calcifying odontogenic cyst

The canonical Wnt pathway activation involves binding of Wnt ligands to the frizzled receptors and the co-receptor LRP-5/6. Once Wnt binds to the

receptor complex, a signal is transduced to the cytoplasmic phosphoprotein DVL-1 and it induces events that disrupt the multiprotein “destruction complex” of Beta-catenin (encoded by *CTNNB1*), formed by Axin-1, APC, GSK3B, and CKI-alpha. Once stabilised and accumulated in the cytoplasm, Beta-catenin is translocated into the nucleus and exerts its effect by binding with the transcription-factors TCF/LEF-1, promoting downstream gene expression [98] (Figure 2). Beta-catenin accumulation in the cytoplasm and nucleus is a hallmark of canonical Wnt pathway activation. Abnormal activation of the Wnt pathway is associated with the pathogenesis of several types of cancer [99] and odontogenic lesions, especially COC.

In a study in 2003, an abnormally-strong nuclear and cytoplasmic Beta-catenin immunostaining was reported in 6/6 cases of COC [100]. Subsequently, *CTNNB1* DNA sequencing with primers spanning the phosphorylation site for GSK3B revealed genetic mutations in 10 COC samples [101]. Nuclear co-expression of LEF-1 and Beta-catenin were also reported in COC [102]. In 2016, de Sousa and her colleagues simultaneously interrogated ~2800 somatic mutations in 50 oncogenes and tumour suppressor genes in three COC cases [103]. The only pathogenic genetic alteration found was in *CTNNB1*, supporting the concept that constitutive activation of the Wnt signalling pathway through Beta-catenin mutation is the pivotal event underlining the COC tumorigenesis.

Besides COC, the dentinogenic ghost cell tumour (DGCT) and the ghost cell odontogenic carcinoma (GCOC) were found to harbour *CTNNB1* genetic alterations at different codons [104,105]. Furthermore, the nuclear and cytoplasmic Beta-catenin accumulation were observed in all these lesions by

immunohistochemistry [105], indicating that aberrant signalling in odontogenic epithelium coordinated by Beta-catenin plays a role in their pathogenesis. Notably, COC, DGCT and GCOC can form dentinoid material or odontoma-like structures (Figure 1). The formation of dental hard tissues is intimately associated with the crucial role of Wnt signalling pathway in inducing tooth formation. For example, the Wnt activation by *Ctnnb1* mutation or *Apc* deletion in mouse's oral epithelium generated ectopic teeth formation [106–108]. Furthermore, the constitutive activation of Beta-catenin in dental epithelium was shown to lead to the formation of odontoma-like lesions with osteodentine and dysplastic dentine in mice jaw, as we detail later [93].

Wnt/Beta-catenin signalling is considered a potent regulator of calcified tissue development and homeostasis in many contexts, such as bones and teeth. Over-activity of Wnt signalling caused by persistent Beta-catenin stabilisation in the dental mesenchyme induced excessive dentin and cementum formation [109]. Conversely, Wnt pathway inhibition by overexpression of Dkk-1 in mice odontoblasts impaired dentin formation in mandibular molars [110]. An attractive hypothesis is that the over-activity of mutated Beta-catenin in epithelial cells of COC might cause aberrant signalling networks of communication with stromal cell compartments, resulting in the production of abnormally calcified dentin-like material, which explains odontoma formation in this lesion. In fact, the paracrine induction of surrounding tissues induced odontoma-like lesions in mice conditionally mutated for Beta-catenin [93]. In this enlightening study, a non-degradable form of Beta-catenin was conditionally expressed in mice in a *Sox2*-expressing subset of dental epithelial stem cells after the cessation of normal tooth induction. Remarkably, these mice

showed calcified structures that histologically resembled the odontomas. The odontoma-like structures had only a partial contribution of cells derived from the epithelium sustaining a Beta-catenin mutation, suggesting that the tumour is also derived from adjacent wild-type mesenchymal tissue [93].

The Wnt ligand WNT5A is also related to the development of dental hard tissue and odontogenic lesions. *Wnt5a*-deficient mice exhibited reduced levels of cell proliferation in the dental epithelium and delayed odontoblast differentiation [111]. In human dental papilla cells, WNT5A overexpression increased the formation of calcified nodules [112]. Interestingly, WNT5A overexpression was reported in COC [113] and ameloblastomas [114]. In enamel epithelium cultured cells, WNT5A overexpression resulted in tumorigenic properties, such as growth factor independence [114], suggesting a role of WNT5A in the biology of ameloblast-like cells in odontogenic tumours.

Ghost cells are a common histological finding in COC. These cells may accumulate enamel-related proteins and undergo calcification [115]. Interestingly, COC ghost cells express WNT1 and WNT5A ligands, as well as Beta-catenin [116,117]. In immature odontomas, ghost cells showed weak Beta-catenin immunostaining, while adjacent epithelial cells exhibited a strong pattern in the nucleus and cytoplasm. Moreover, a positive staining for LEF-1 was found in the cytoplasm of the ghost cells and in the nucleus of adjacent odontogenic epithelial cells [118]. These results provide further support to the idea that the Wnt signalling is involved in the formation of the ghost cells.

Odontogenic lesions, mainly COC, are of particular interest, as they are well known to have striking histological similarities with the brain tumour

craniopharyngioma. This can be attributed to their common embryologic origin from oral ectoderm [119]. The calcified material, ghost cells, and the *CTNNB1* mutation are common findings in the adamantinomatous tumour subtype, which frequently mimics COC histology [119,120]. Conversely, the papillary craniopharyngioma subtype is highly correlated with recurrent BRAFV600E mutations and lacks ghost cells and calcifications [120]. These observations further strengthen the link between Wnt/Beta-catenin pathway activation and formation of ghost cells and calcified structures.

Taken together, Beta-catenin plays a role in the tumorigenesis of COC. Also, the association of abnormal Wnt signalling with ghost cells and dental hard-tissue pathobiology, including odontoma formation, have provided a clue to the mechanism underlining the histopathogenesis of COC and tumours containing ghost cells.

Concluding remarks

Benign lesions can share driver oncogenic mutations that were previously thought to be exclusive to malignant neoplasms. The fact that these benign conditions virtually never convert to a malignant disease, even in the presence of such pathogenic mutations, is intriguing. The understanding of the tumorigenic process and mechanisms that confine cysts and tumours to a benign state should be explored in each organ’s context for cancer prevention and therapy.

Tumours should, therefore, be considered as an abnormal organ. Odontogenic cysts and tumours are abnormal tooth organs, and here we have

reviewed their main genetic alterations (for those lesions already studied) in the context of odontogenesis. Odontogenesis is governed by sequential and reciprocal epithelial-mesenchymal interactions, and as a reflection of that, the induction of a stromal component by mutated dental epithelium plays a role in the formation of odontogenic lesions. The use of cell culture models that mimic all the lesion compartments is urged for the unbiased study of these entities.

In addition, we must consider the developmental setting and the genomic landscape to better understand the tumorigenic role of central signalling, such as MAPK, Shh and Wnt pathways. As odontogenic cysts and tumours probably arise due to developmental defects, and no environmental mutation sources have been identified as a causative for them, they probably harbour quiet genomes similar to some paediatric tumours.

Different odontogenic cysts and tumours arise from different stages of dental lamina cells, as evidenced by their histopathologic features that resemble the embryologic patterns seen in the developing tooth. Pathogenic mutations affecting the same pathway are seen in different tumours, reinforcing the idea that the cell of origin, including its location in the jaws, is dominant to oncogenic signalling in these kinds of tumours.

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Statement of author contributions

MGD, CCG, SFS, GMX drafted the article. MGD, CCG, and RSG critically revised its intellectual content; MGD conceptualized and designed the figures; all authors reviewed and approved the final manuscript.

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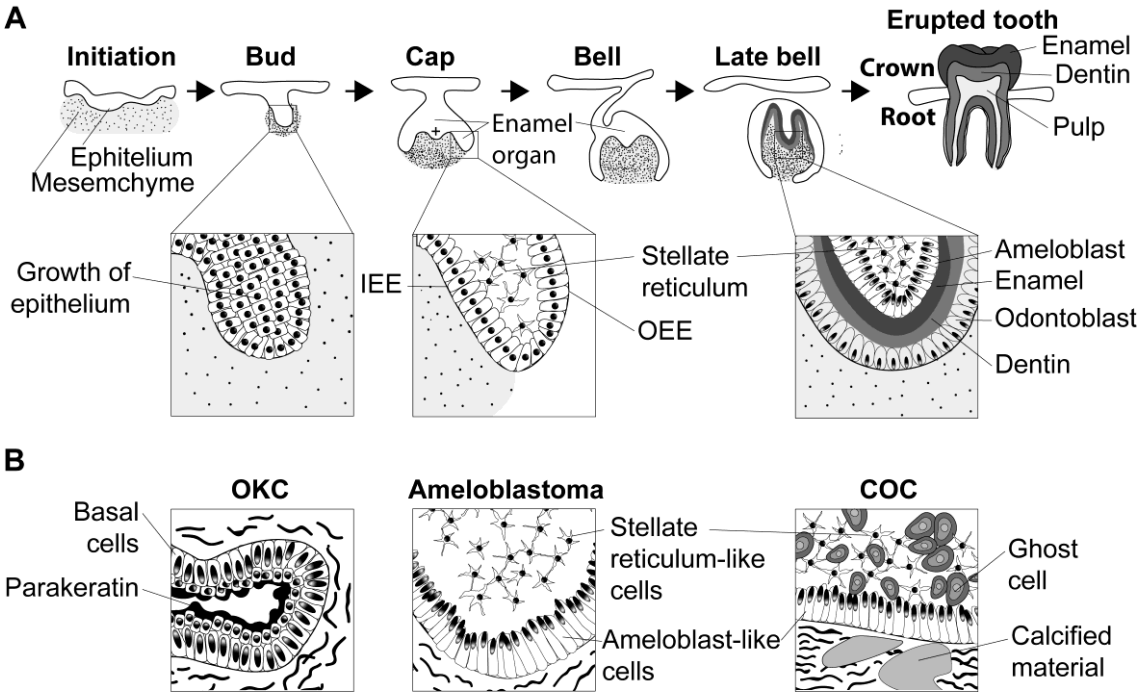

Figure 1. Schematic illustration of odontogenic cysts and tumours histopathologic features that resemble, in part, embryologic patterns seen in the developing tooth. (A) Tooth development is divided into initiation, bud, cap, and bell stages, followed by root formation and eruption. At initiation, epithelial thickening invaginates into the underlying mesenchyme to form a bud shape. In the cap stage, the enamel knot (+), and the enamel organ are formed. The stellate reticulum consists of star-shaped epithelial cells and is formed in the middle of the enamel organ, between inner and outer enamel epithelium (IEE and OEE, respectively). During the bell stage, ameloblasts differentiate from the IEE and odontoblasts from the dental mesenchyme (dental papilla). Later, deposition of the extracellular matrices of dentin and enamel begins. (B) Odontogenic keratocyst (OKC) epithelium is characterized by a parakeratinized-stratified squamous epithelium with columnar epithelial cells at basal layer. OKC arises from cells of the early stage of odontogenesis before enamel organ

formation has taken place, and sometimes it might form from tooth bud instead of a tooth. Ameloblastoma and calcifying odontogenic cyst (COC) are lesions of the enamel organ and present ameloblast-like cells with reversely polarised nuclei and stellate reticulum-like cells. While in ameloblastomas there is no induction of hard-tissue formation, COC can present calcification (dentinoid-like material, adjacent to epithelial component and dystrophic mineralisation of ghost cells).

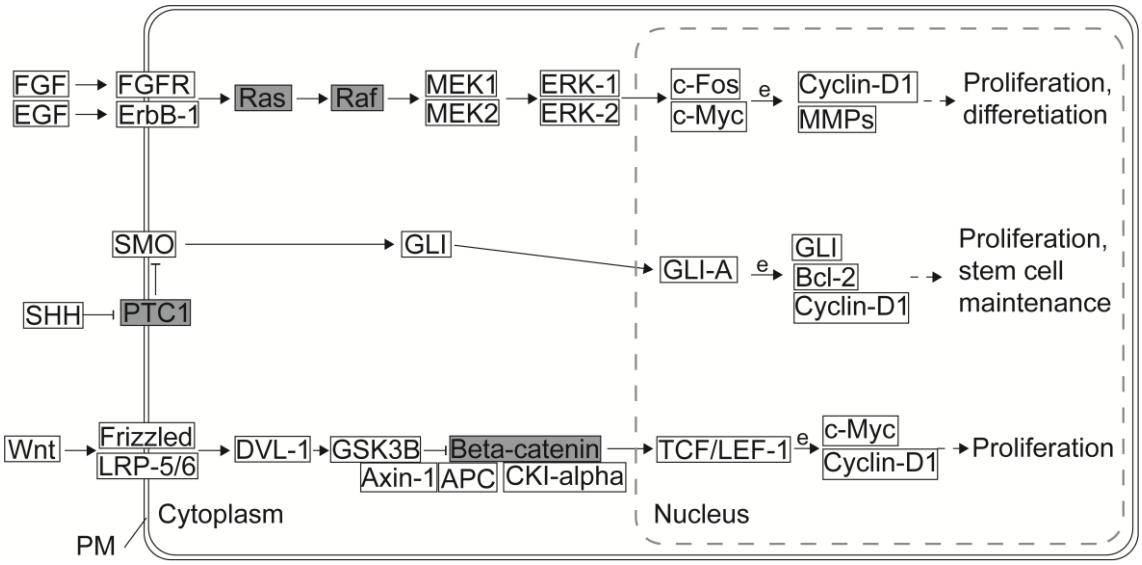

Figure 2. Cell signalling alterations in odontogenic cysts and tumours. MAPK, Shh and Wnt signalling pathways play a role in every step of normal tooth development, and genetic alterations affecting these pathways (grey boxes) occur in a set of odontogenic cysts and tumours. Activating mutation in the Ras GTP-binding protein (*KRAS*) and Raf kinase (*BRAF*) in adenomatoid odontogenic tumour and ameloblastoma, respectively, results in over activity of ERK-1/2 kinase in cytoplasm and nucleus. Inactivation of the transmembrane receptor PTC1 in odontogenic keratocyst results in the constitutive production of the active form of transcription factor GLI (GLI-A), increasing the expression of

target genes. Gain-of-function mutations in Beta catenin (*CTNNB1*) in COC result in stabilisation, translocation to the nucleus, and activation of transcription. Ultimately, alterations of these pathways in odontogenic cysts result in increased cell proliferation. PM, plasmatic membrane; e, expression.

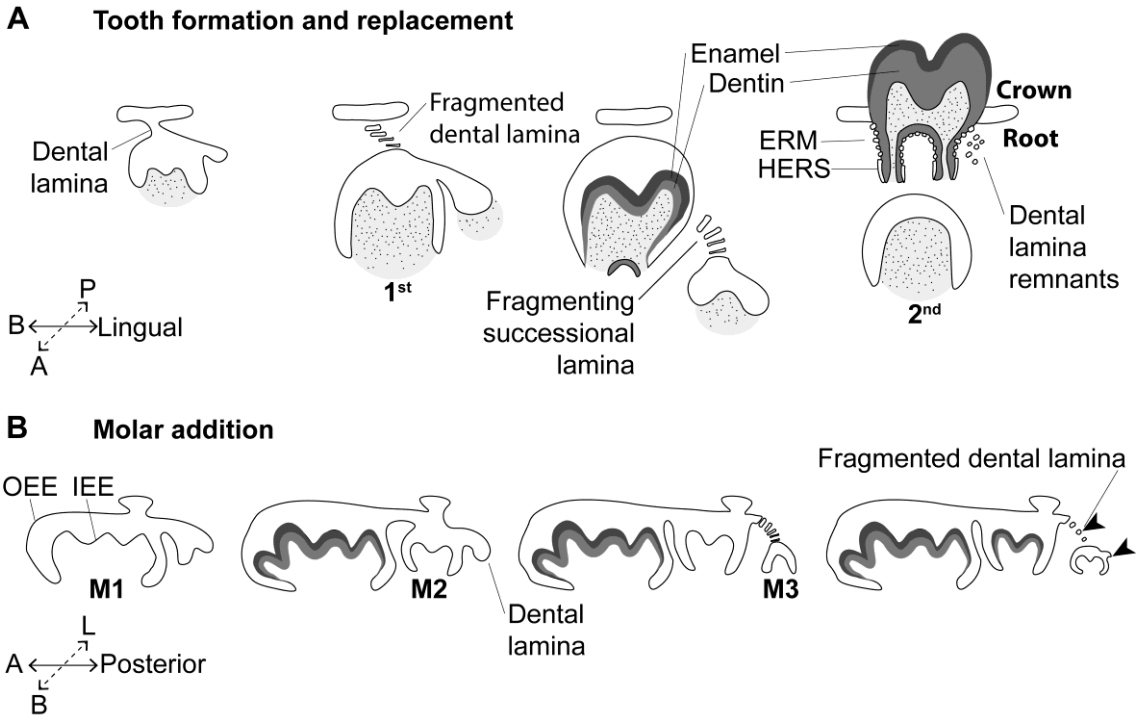

Figure 3. Schematic illustration of cells' source for tooth development and odontogenic cysts and tumours. Odontogenic cysts and tumours arise from cells that participate in tooth formation and replacement. (A) At the cap stage of the deciduous tooth (1st), the dental lamina on the lingual side (successional dental lamina) grows into the underlying mesenchyme towards the apex of the deciduous tooth (2nd). Later, the dental lamina connecting the primary teeth to the oral epithelium and the successional lamina connecting the deciduous and the permanent teeth are degraded, forming dental lamina remnants. Root development is guided by Hertwig's epithelial root sheath (HERS) that fragment when the tooth erupts, forming the epithelial cell rests of Malassez (ERM) on

the root surface. (B) The permanent molars form posteriorly to the deciduous teeth and are not replaced. The primary molars erupt in a specific sequence (M1, molar 1; M2, molar 2; M3, molar 3) from the posterior extension of the dental epithelium. Dental lamina and its fragment cells expressing SOX-2 are associated with the development of third and fourth molars and with odontogenic cysts and tumours formation (arrowheads).